THE EFFECTS OF HYDROCORTISONE ON THE BLOOD OF TADPOLES AND FROGS, RANA CATESBEIANA

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In his 1957 monograph, Chester Jones stated, "Little information is available from the results of the injection of adrenocortical steroids, used in mammalian work, into Amphibia" (pp. 153–154). Since that time, various observational and experimental attacks on the anterior pituitary-adrenocortical system of the Amphibia have increased our understanding of the adrenal cortex, its role and its control in this group of vertebrates. Aldosterone and corticosterone have been identified as the major cortical steroids of the anurans (Carstensen, Burgers and Li, 1961; Hanke and Weber, 1964; Pesonen and Rapola, 1962; and Phillips and Bellamy, 1963), and recently, Piper and de Roos (1967) have found that both these corticoids have negative feedback influence on the pituitary's secretion of ACTH in *Rana catesbeiana*. Hydrocortisone, an obvious product of the adrenal cortex of mammals, seems to be of lesser significance in the amphibians, although it has been found in cortical extracts or in excretory products of representatives of several genera of this group (Chester Jones, Phillips and Holmes, 1959; Dale, 1962; and Pesonen and Rapola, 1962). Further, studies in our laboratory have established that responses to injections of this hormone do occur in *Rana pipiens* (Bennett and Alspaugh, 1964; and Bennett and Newell, 1965). Changes in the distribution of leucocytes, similar to those seen in mammals and reported originally by Dalton and Selye (1938), were found as early as 12 hours after and as late as 144 hours after the treatment of frogs with hydrocortisone acetate. Of what significance these reactions are in the normal economy of *R. pipiens* is not known. The possible stress imposed by dehydration did not effect lymphopenia and neutrophilia in these frogs as did the injection of hydrocortisone (unpublished observations).

Among the many other questions we have regarding the role of hydrocortisone in Amphibia are these: does this steroid evoke changes in the blood picture of species other than *R. pipiens;* does it have effects on the distribution of white blood cells in immature as well as mature anurans? These are the questions which this study attempted to answer.

Materials and Methods

The tadpoles, *R. catesbeiana,* were obtained from a supplier in North Carolina in October, 1967. They were kept in battery jars of pond water at a temperature which ranged between 9.5° and 10.5° C. Fifty tadpoles were studied. The experimental animals, 25, were injected with 0.5 cc. of a 0.2% suspension of hydrocortisone acetate (Nutritional Biochemical Corporation) in distilled water, and the controls, 25, were injected with the same amount of distilled water. The injections were made into the musculature of the tail in all cases. Forty-eight hours after injection, blood was obtained from the tail; smears were stained with Wright's blood stain. Differential counts of 100 leucocytes were made for each animal.

Frogs, *R. catesbeiana*, were sent from a supplier in Wisconsin from October through December 1967. These animals were kept in individual battery jars containing about 2 cm. of tap water in a room in which the temperature varied between 9.5° and 10.5° C. The 24 experimental animals received 1 cc. of a 0.2% suspension of hydrocortisone acetate in distilled water while 24 controls were injected with 1 cc. of distilled water. The injections were made under the skin of the ventrolateral surface of the posterior trunk. Again, blood samples were withdrawn 48 hours after treatment. Smears were stained with Wright's stain, and differential counts of 100 white cells were made for each frog.

RESULTS AND DISCUSSION

The average per cent distributions of neutrophils and lymphocytes for the control and experimental animals are given in Table I. Also included in this table are the Student's "t" values for the differences between the two types of blood cells for the controls and experimentals. The difference between the number of lymphocytes in the control and experimental tadpoles was found to be significant ($P < 0.025$), as was the difference between the number of neutrophils in these two groups of animals ($P < 0.01$). The number of lymphocytes in the control frogs was much greater than in the experimentals, and again, the difference was significant ($P < 0.005$). The difference between the counts of neutrophils in these two groups of frogs was also significant ($P < 0.01$).

TABLE I

The average per cent distribution of lymphocytes and neutrophils and the Student's "t" values for the differences between counts of the two types of cells for control and experimental tadpoles and frogs

Animals	% Lymphocytes	"t"	% Neutrophils	"t"
Experimental tadpoles	30.08		49.82	
Control tadpoles	58.12	2.07	21.48	2.44
Experimental frogs	17.50		70.71	
Control frogs	57.46	3.10	33.46	2.23

Thus, it is evident that both the immature and mature forms of *R. catesbeiana* responded to the hydrocortisone as do mature *R. pipiens*. The directions of the responses, *i.e.*, an increase in neutrophils and a decrease in lymphocytes, were also the same in the three groups of animals. However, the average intensity of the reactions varied. In the experimental tadpoles, the neutrophils increased 131% over those of the controls while the lymphocytes decreased 49%. For the experimental frogs, *R. catesbeiana*, the increase in neutrophils was 111%, and the decrease in lymphocytes was 69%. Forty-eight hours after the injection of the same amount of hydrocortisone as was used in the present study, the number of neutrophils was

95% greater and the number of lymphocytes was 41% lesser in mature, experimental *R. pipiens* than in their controls (Bennett and Newell, 1965).

The significant decreases in monocytes, eosinophils and basophils which were observed 72 and 144 hours after administration of comparable doses of hydrocortisone to *R. pipiens* (Bennett and Alspaugh, 1964) were not seen in the blood of *R. catesbeiana* or of adult *R. pipiens* 48 hours after injection of the steroid (Bennett and Newell, 1965). The distribution of these rarer leucocytes may change only after more than 48 hours of exposure to hydrocortisone. However, the distribution of leucocytes in normal frogs varies widely; thus valid generalizations about even the *typical* white blood cell picture are difficult to state (Schermer, 1967). The differences in intensity of neutrophilia and lymphopenia reported here, as well as the differences between the counts of monocytes, basophils and eosinophils of experimental *R. catesbeiana* and *R. pipiens* at 48 hours and those at 72 and 144 hours after treatment, are in reality only slight and are probably not of biological significance.

The increase in neutrophils and the decrease in lymphocytes in the two species of frogs are certainly significant, and are of interest, especially since they parallel those well known in mammals in which titers of hydrocortisone have been increased by physiological stress or injection. The review of Selye (1950) continues to provide us with details of these changes in mammals. The exact manner in which some of the adrenal steroids effect the responses observed in the blood continues to be problematical. Also not fully understood is the role of the adrenocortical steroids in the immature and/or metamorphosing anuran. Frieden and Naile (1955) reported that hydrocortisone accelerated metamorphosis of thyroxin-treated *R. pipiens* and *R. hechsheri.* The histochemical studies of Rapola (1963) on *Xenopus laevis* also implicate adrenal steroids in metamorphic phenomena. Our work proves only that immature *R. catesbeiana*, as well as mature frogs of this species, do have the system which causes the blood picture of the animals to respond to hydrocortisone as does mature *R. pipiens.* What the mechanism responsible for these changes is; whether it operates in response to intrinsic steroids; whether it is of biological value to these amphibians remain unknown.

Summary and Conclusions

1. Differential counts of the leucocytes of tadpoles and frogs, *R. catesbeiana*, were made 48 hours after the injection of hydrocortisone acetate (experimentals) or distilled water (controls).

2. Neutrophilia and lymphopenia were evident in both the immature and mature experimental animals but not in the controls.

3. These changes in the distribution of white blood cells resemble those known in mammals and in *R. pipiens.*

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